

A major suppressor of cell death, *slm1*, modifies the expression of the maize (*Zea mays* L.) lesion mimic mutation *les23*

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Abstract: Disease lesion mimics provide an excellent biological system to study the genetic basis of cell death in plants. Many lesion mimics show variation in phenotype expression in different genetic backgrounds. Our goal was to identify quantitative trait loci (QTL) modifying lesion mimic expression thereby identifying genetic modifiers of cell death. A recessive lesion mimic, *les23*, in a severe-expressing line was crossed to the maize inbred line Mo20W, a lesion-suppressing line, and an F₂ population was developed for QTL analysis. In addition to locating *les23* to the short arm of chromosome 2, this analysis detected significant loci for modification of lesion expression. One highly significant locus was found on the long arm of chromosome 2. The Mo20W allele at this QTL significantly delayed initiation of the lesion phenotype and decreased the final lesion severity. Other QTL with lesser effect affected severity of lesion expression without affecting lesion initiation date. Our results demonstrate that dramatic change in lesion phenotype can be controlled by a single major QTL. The presumed function of this QTL in normal plants is to regulate some aspect of the cell death pathway underlying the *les23* phenotype.

Key words: genetic background, quantitative trait locus, phenotype suppression, Mo20W, corn.

Résumé : Les mutations qui miment la réaction hypersensible constituent un excellent système biologique pour étudier les bases génétiques de la mort cellulaire chez les plantes. Plusieurs de ces mutants montrent une variabilité phénotypique selon le fond génétique dans lequel elles se retrouvent. Le but de ce travail était d'identifier des locus à caractère quantitatif (QTL) qui modifient l'expression de ces mutations afin d'identifier les modificateurs de la mort cellulaire. Une lignée portant une mutation récessive, *les23*, et conférant un phénotype marqué a été croisée à la lignée fixée Mo20W, laquelle entraîne une suppression des lésions, et une population F₂ en a été dérivée pour fins d'analyse QTL. En plus de permettre la localisation de *les23* sur le bras court du chromosome 2, cette analyse a mis en lumière des locus influençant de manière significative l'expression de lésions. Un locus hautement significatif a été trouvé sur le bras long du chromosome 2. L'allèle Mo20W à ce locus entraînait un retard important dans l'apparition des lésions et une diminution de la sévérité des lésions. D'autres QTL à effet moindre affectaient la sévérité des lésions mais sans en altérer la date d'apparition. Ces résultats montrent qu'un changement dramatique au niveau du phénotype observé peut être déterminé par un QTL unique majeur. La fonction présumée de ce QTL chez les plantes normales est de réguler un quelconque aspect du sentier de mort cellulaire qui sous-tend le phénotype *les23*.

Mots clés : fond génétique, locus à caractère quantitatif, suppression du phénotype.

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Introduction

Lesion mimics are a class of mutants that often exhibit symptoms resembling disease without a causal agent present (Johal et al. 1985; Jambunathan et al. 2001; Gray et al. 2002; Balague et al. 2003). Although initially identified in

maize, such mutants have been found in all plant species examined. The lesion mimic phenotype is characterized by patches of dead cells on plant parts and is often inherited as a simple mutation that can be dominant, partially dominant or recessive (Neuffer and Calvert 1975; Johal et al. 1985; Balague et al. 2003). Lesion mimics can be separated into

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two classes: initiation lesion mimics where lesions initiate extensively, but stay discrete in size, and propagation lesion mimics where lesions initiate rather sparingly but, once formed, expand continuously to engulf the entire tissue (Johal et al. 1985; Dangl et al. 1996).

Genes underlying a number of lesion mimics have been cloned recently, revealing the involvement of multiple pathways or processes that are responsible for the lesion mimic phenomenon. For example, some lesion mimics result from structural aberrations in *R* genes that confer gene-for-gene specific resistance during plant-pathogen interactions (Pryor 1987; Hu et al. 1996; Shirano et al. 2002). Some result from disruption of homeostasis that may accrue from errors in various metabolic pathways (Hu et al. 1998; Mach et al. 2001). Still others arise from alterations in genes that may play key functions in pathway(s) leading to programmed cell death (PCD) (Dangl et al. 1996; Gray et al. 1997, 2002; Brodersen et al. 2001; Liang et al. 2003). However, one attribute that seems to tie most mimics together is the involvement of free radicals in the ontogeny of lesions associated with these mutations (Dangl et al. 1996; Dietrich et al. 1997; Hu et al. 1998; Mach et al. 2001; Balague et al. 2003).

Although lesion mimics are caused by single gene mutations, their phenotype is influenced by a number of factors both external and internal. While some mimics have specific requirements for temperature and relative humidity for manifestation, most appear to require light, again reflecting the importance of reactive species in lesion formation and (or) propagation in these mutants (Hoisington et al. 1982; Johal et al. 1985; Dietrich et al. 1997; Hu et al. 1998; Brodersen et al. 2001; Jambunathan et al. 2001; Mach et al. 2001; Gray et al. 2002).

Among the internal factors that affect lesion mimics are the plant age (developmental stage) and genetic background. Most lesion mimics are developmentally programmed; lesions only form when plants reach a certain developmental or physiological age. What dictates this behavior of lesion mimics remains unknown, although it can be affected to varying degrees by the genetic background of the plant. In addition, genetic background has also been shown to dramatically influence the severity of lesions with most lesion mimics in maize. The same lesion mimic may have a lethal phenotype in one genetic background, but a largely benign phenotype in another. Maize inbred lines have been found that either suppress or enhance the phenotype of multiple lesion mimics. For example, Mo20W suppresses many, but not all, lesion mimics, whereas W23 provides an optimal background for severe expression. The suppressible factor(s) from Mo20W appears to be dominant because F_1 plants derived from Mo20W crossed to the *Les1* mutant in a W23 background showed both a delay in initiation of lesions as well as a reduction in the size and frequency of lesions compared with the *Les1* mutant in a W23 background (Neuffer et al. 1983).

The lesion mimic described in this study, *les23* is a recessive mutant whose chromosomal location had not been determined. In a permissive genetic background, *les23* forms early lesions that show slight expansion, but mainly coalesce into large lesions through repeated initiation of new lesions. It had been observed that *les23* lesion expression was sensitive to genetic backgrounds such as Mo20W. In a pilot study,

individual (Mo20W \times *les23*) F_2 plants homozygous for the *les23* mutation showed a wide array of lesion phenotypes for initiation date, extent of leaf covered with lesions, and overall severity of lesions. These results indicated that this population could serve as an appropriate basis for a quantitative trait locus (QTL) study on the effects of genetic background on lesion mimic expression.

A QTL analysis of lesion expression in a sub-population of (Mo20W \times *les23*) F_2 plants homozygous for the *les23* mutation was conducted in two locations in different years to determine the genetic control of lesion expression. The (Mo20W \times *les23*) F_2 population was also used to determine the genetic location of the *les23* locus.

Materials and methods

Plant materials and phenotype data collection

The *les23* mutant, originally called *wilted-spotted*-74-1873-9*, was generously provided by Phillip Stinard, Maize Genetics Cooperation Stock Center, Urbana, Ill. The recessive mutant originated in an ethyl methane – sulfonate mutagenesis screen of an *opaque* version of the inbred line Va35 and was maintained by repeated sib mating of the homozygous mutant plants with wild-type heterozygous plants. To generate progeny for the QTL analysis, pollen from *les23* mutant plants was used to pollinate the inbred line Mo20W. The resulting F_1 plants were self-pollinated to generate a large population of (Mo20W \times *les23*) F_2 individuals. The F_2 population was used to test for QTLs in two different environments in two different years (Iowa in 1999 and Missouri in 2001). The Iowa 1999 (IA99) and Missouri 2001 (MO01) experiments consisted of two separate groups of F_2 plants from 10 self-pollinated F_1 ears.

The IA99 experiment consisted of 512 individuals that were identified as homozygous *les23* in the (Mo20W \times *les23*) F_2 population grown at Pioneer Hi-Bred International, Johnston, Iowa, during the summer of 1999. This represents approximately 1/4 of the 2000 individuals, as would be expected for segregation of a single recessive mutation. The first 374 lesion-expressing individuals by row position were used in the QTL analysis. The date of initial lesion formation (initiation) was recorded for each plant approximately every 5 days starting 30 days after planting. A final severity rating of the plants was taken when a majority of the plants were just entering senescence. Severity was assessed based on leaf area covered by lesions, the number of leaves covered by lesions, and the presence or absence of necrotic spots and lesions with purple borders. A scale of 1–10 was used based on visual inspection of each plant, with 1 corresponding to extremely mild chlorotic spots on a few leaves and 10 being a dead and collapsed plant (Fig. 1, panels A1–A10). Visual estimates of percent lesion coverage of the 7th and 10th leaves were made at the same time.

The MO01 experiment consisted of 330 individuals that were identified as homozygous *les23* in the (Mo20W \times *les23*) F_2 population grown at the Bradford Research and Extension Center, Columbia, Missouri, in the summer of 2001. This represents approximately 1/4 of the 1350 F_2 plants. Of the expressing individuals, the first 189 by row position were used in the QTL analysis. Lesion initiation date was recorded every 2–3 days. Beginning after day 60, symptom se-

Fig. 1. Images of the *les23* phenotype. (A1–A10) Typical final severity rating classes of F_2 plants observed in the field for the IA99 experiment taken just before most plants started to enter senescence. The 10 leaf is shown in A1–A8. The classes are arranged in order from 1 to 10. (B1–B3) Photos of the three stages of the development of the *les23* lesion mimic. (B1) Initiation. (B2) Progression. (B3) Full development where non-expressing siblings are still green, but lesion mimics are almost completely senescent. (C1 and C2) Photos of *les23* light dependence. Panel C1 shows a leaf from a *les23* mutant plant that had been covered with foil prior to lesion expression. Panel C2 shows the area underneath the foil where lesions did not form.



verity rating as described for the IA99 experiment and the 10th leaf lesion coverage were recorded every 3–4 days.

Light dependency of *les23*

Sections of the top-most fully emerged leaves of *les23* plants 4–6 weeks after emergence were covered with aluminum foil, with or without a blue or red color filter, before the formation of visible lesions (Hu et al. 1998; Gray et al. 2002). These experiments were carried out in the greenhouse in winter 2002 and again in the field in the summer of 2003. After lesions formed on the uncovered sections of the leaves (approximately 3 weeks), the foil was removed and presence or absence of lesions was recorded.

Genotype determination

For the IA99 and MO01 experiments, a single leaf of plant tissue was collected and lyophilized early in the season. Approximately 400 mg of ground dried tissue was extracted by a mixed alkyltrimethylammonium bromide method based on Saghai-Marooof et al. (1984). Polymerase chain reactions (PCRs) were performed in 96-well plates with a total volume of 20 µL of the following mix: 40 ng of sample DNA, 1× Jumpstart *Taq* mix (2.5 mM MgCl₂, 0.4 mM each of dNTPs, 0.3 U Platinum *Taq*; Sigma, St. Louis, Mo.), and 50 ng each of forward and reverse primers. The PCR conditions were as follows: (i) 10 cycles of denaturation at 94 °C for 1 min, annealing for 1 min at 65 °C, decreasing the temperature by 1 °C per cycle, and extension at 72 °C for 1.5 min; (ii) 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min 30 s. The PCR products were stored at 4 °C until used. The PCR was carried out on either an MJ Research PTC 225 (Waltham, Mass) or a Thermolyne AmpliTron II (Dubuque, Iowa) thermalcycler. The maize simple sequence repeat (SSR) marker set (MaizeGDB, www.maizegdb.org/ssr.php) was tested against the parents and F₁s of the (Mo20W × *les23*)F₂ population to identify polymorphic markers covering the entire genome at a distance of approximately 20 centimorgans (cM) between markers. The genotypes of 374 individuals in the IA99 experiment and 189 individuals in the MO01 experiment were determined for 103 SSR markers by electrophoresis of the PCR products on 4% superfine resolution agarose gels (Amresco, Solon, Ohio). Digital images of each gel were recorded and genotypes were classified by visual inspection of the PCR products.

Map construction and QTL analysis

Linkage maps were constructed with Mapmaker/EXP version 3.0b (Lander et al. 1987). The group command was used to form linkage groups, and each was assigned to a chromosome based on current genetic maps of maize (Davis et al. 1999; Sharopova et al. 2002). Marker order and distances were determined by the order command. The linkage maps for the IA99 and MO01 experiments had 96 and 98 informative markers placed on 10 chromosomes, respectively. The marker orders and distances were consistent with published maize genetic maps (Davis et al. 1999; Sharopova et al. 2002).

Estimates of QTL position and genetic effects were determined using QTL Cartographer version 1.16c (Basten et al. 1994, 1999). The Rmap and Rcross commands were used to

translate the Mapmaker files to Cartographer format. The SRmapqtl command was used to identify QTL co-factors selected by a forward–backward stepwise regression with thresholds for inclusion and exclusion at $P \leq 0.01$. The Zmapqtl command was used to search for QTL by composite interval mapping (Model 6) using a window size of 10 cM, interval size of 2 cM, and all parameters selected by the SRmapqtl command. Permutation analysis was performed on the data sets using QTL Cartographer. Based on a permutation analysis of 1000 randomized QTL data sets, the likelihood of odds (LOD) score of 3.5, corresponding to a 95% experiment-wise significance threshold, was used to declare a QTL significant.

Statistical test for rate of lesion progression

Linear regression analysis was performed on the lesion progression rate of the MO01 experiment between 68 and 82 days after planting for plants classified by genotypes at *bnlg1045* using the PROC REG command in SAS (SAS Institute, Inc. 1999). Also, 95% confidence intervals for each value were calculated by using PROC ANOVA in SAS using genotypes at *bnlg1045* as the “class” in the following MODEL statement: day = *bnlg1045*.

Results

Phenotype of *les23* lesions

In the Va35 background, *les23* mutants exhibit a fairly uniform phenotype with a severity that roughly corresponds to a rating of 8 in Fig. 1, panels A1–A10. In this relatively permissive background, *les23* lesions initiate around 40–45 days after planting if grown in the field, or within 35 days if grown under greenhouse conditions (Fig. 1, panel B1). The lesions first form in the oldest (lowest) leaves, usually near the tips of the leaves. These lesions grow slightly and turn necrotic, slowly outgrowing the brown to purplish borders (black arrows) formed around the initial lesions (Fig. 1, panel B2). As the plants age, additional lesion formation can cover the leaves, thereby causing early senescence (Fig. 1, Panel B3). With time, lesions appear on progressively younger leaves and repeat the process of early leaf senescence.

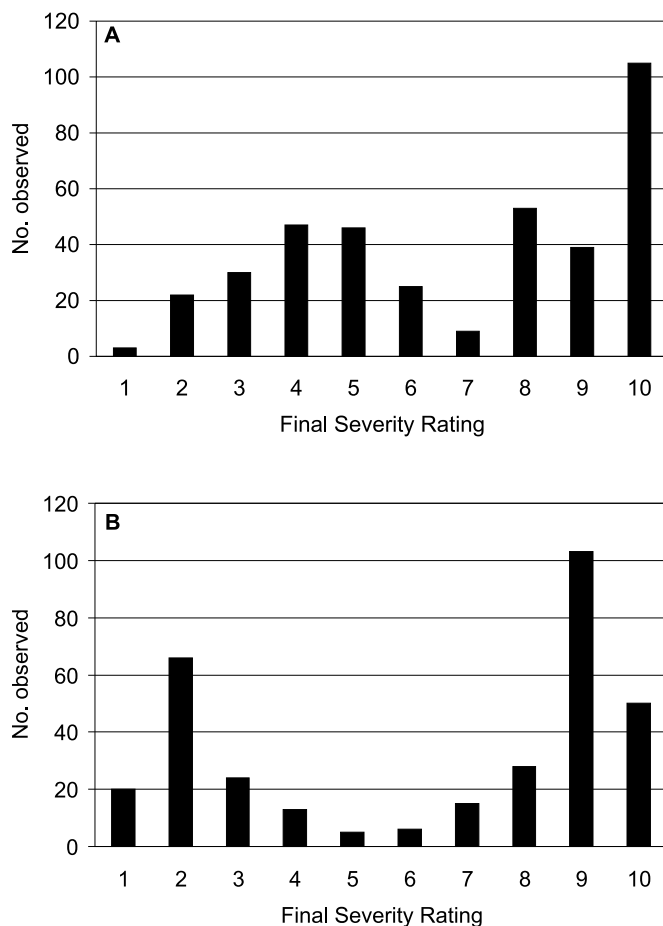
les23 lesion formation is light dependent

Light dependency of lesion formation was demonstrated by covering leaf sections with aluminum (Fig. 1, panels C1 and C2). A few small lesions sometimes formed in the foil-covered area closest to the tip of the leaf on older leaves; otherwise, lesions failed to develop in areas of the leaf protected from light. The dependency of lesion formation on specific wavelengths of light was tested using blue and red filters. Lesions were not formed under either the blue or red filters, which suggests that quantity, not quality, of light may be essential for lesion formation.

Phenotype expression of (Mo20W × *les23*)F₂ plants

To view expression of the *les23* phenotypes under different environmental conditions, two experiments were conducted; one in Iowa during the summer of 1999 (IA99) and one in Missouri during the summer of 2001 (MO01). Of the 2000 individuals in the IA99 experiment, and of the 1250 individuals in the MO01 experiment, approximately 1/4 of

Fig. 2. Distributions for final lesion severity rating in the two QTL experiments. Classes show increased phenotype severity with each unit increase in final severity rating. Classes 1–5 show increases in percent lesion coverage of the leaf with chlorotic lesions and class 5 contains some stem lesions. Classes 6–10 show increased lesion expression beyond class 5 with necrotic lesions increasing in total percentage of lesions. Many of the higher classifications have stem lesions. Class 9 plants were up-right but completely necrotic. Class 10 plants were completely necrotic, fallen, and had started to decay. (A) IA99 experiment, 374 individuals. (B) MO01 experiment, 189 individuals.



each expressed some form of *les23* lesion phenotype as expected for its recessive inheritance. The timing of lesion initiation and severity of lesion coverage was quite variable among individuals. For plants exhibiting the *les23* phenotype in both the IA99 and MO01 experiments, the final severity ratings displayed a bimodal distribution (Fig. 2). This surprising result indicated that control of lesion severity might have a fairly simple genetic basis.

Determination of map position for *les23*

Because of its recessive nature, modification of the *les23* phenotype can only be observed in *les23/les23* homozygous plants. Therefore, QTL analysis was performed on that subset of the (Mo20W × *les23*)F₂ population expressing some degree of lesion phenotype. Using this expressing sub-population allows for identification of the location of the *les23* gene because genotypes of SSR marker(s) will con-

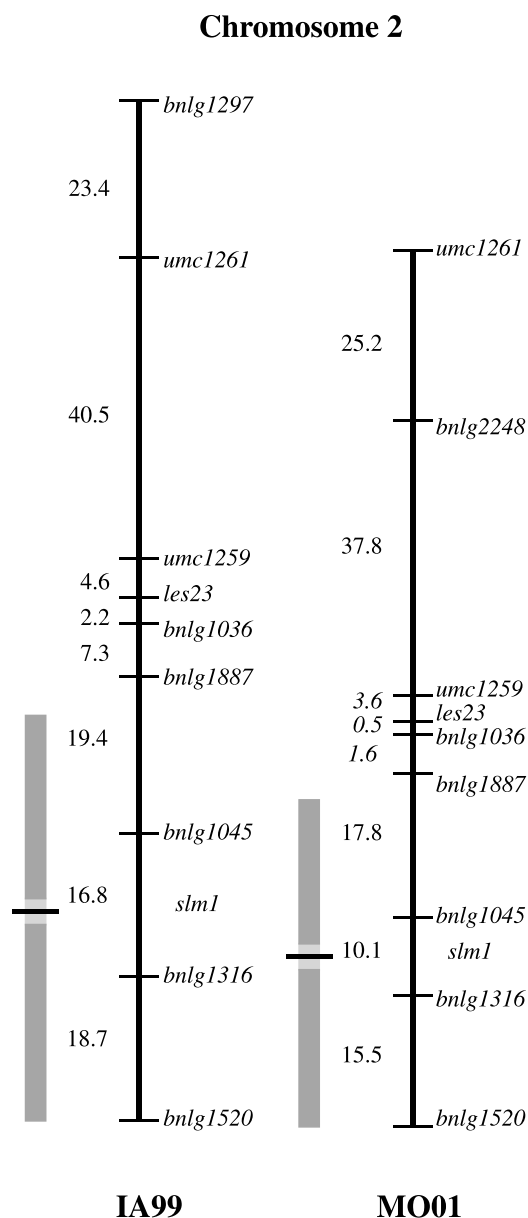
verge to a point of all homozygous *les23* alleles at the position of the *les23* locus. Results from the IA99 experiment suggested the *les23* gene was located on chromosome 2 near *bnlg1036*; almost all individuals expressing a lesion phenotype were homozygous for the *les23* parent allele at that SSR locus. To test this hypothesis, a locus (*les23*) was constructed having only *les23* parent alleles and added to the file of SSR genotypes used by Mapmaker/EXP to generate genetic maps for each of the IA99 and MO01 experiments. As predicted, *les23* was located on chromosome 2, near *bnlg1036* (Fig. 3). To confirm these results, the tissue from the first 200 individuals in the MO01 population, regardless of the *les23* phenotype, was collected and genotypes for all chromosome 2 markers (Fig. 3) were obtained. A map was constructed in Mapmaker with one additional dominant marker based on the phenotype of each plant: *les23/les23* for lesion-expressing plants and Mo20W/- for non-expressing plants. The map position of this phenotypic marker was between *umc1259* and *bnlg1036*, confirming the location of *les23* (data not shown).

Quantitative trait analysis of lesion phenotype

Composite interval mapping was performed using 374 *les23/les23* individuals from the IA99 experiment and separately for 189 *les23/les23* individuals from the MO01 experiment for the traits of lesion initiation date and various measures of lesion severity (Table 1). For both the IA99 and MO01 experiments, a single, very large QTL on chromosome 2 near *bnlg1045* explained almost all the variation in lesion initiation date (Table 1; Fig. 3). This QTL is also the major factor in all measures of lesion severity (Table 1). We have designated the QTL *slm1*, *suppressor of lesion mimics 1*. The LOD values for *slm1* drop below the significance threshold before reaching the position of the *les23* mutation, clearly indicating that *les23* and *slm1* are distinct loci (Fig. 3). While *slm1* was the only QTL detected for initiation date, other minor QTL were detected for the various measures of lesion severity and lesion growth (Table 1). These minor QTL explained much less trait variation and had much smaller gene actions than *slm1* (Table 1).

Because of the linkage of *slm1* with *les23* (Fig. 3) and because only *les23/les23* individuals display a lesion phenotype, there was a biased distribution of genotypes at the *slm1* locus. There were only 4 homozygous Mo20W individuals at the *bnlg1045* locus in each of the IA99 and MO01 experiments. Since determination of additive and dominance effects requires comparison of all three genotype class means, the values for the genetic effects as calculated within QTL Cartographer may have been affected by this biased genotype distribution. Therefore, the genetic effect of the Mo20W allele at the *slm1* locus in modulating initiation date and final lesion severity was calculated by comparing the class means for the *les23/les23* vs. Mo20W/*les23* individuals for *bnlg1045*. In the IA99 experiment, the *les23/les23* class mean for initiation date was 39.2 days after planting (244 individuals) and the *les23*/Mo20W class mean was 55.5 days after planting (122 individuals). This indicates that one Mo20W allele of *slm1* delayed lesion initiation by 16.3 days in the IA99 experiment. Likewise, one Mo20W allele of *slm1* reduced the final severity rating by 3.7 units. In the MO01 experiment, one Mo20W allele of *slm1* delayed initi-

Fig. 3. Linkage map of chromosome 2 for the IA99 and MO01 experiments showing the location of the recessive mutation *les23*, and *slm1*, the major QTL for *les23* phenotype suppression. The distances between markers, in centimorgans (cM), are listed to the left. The dark gray bars indicate the range of the QTL for lesion initiation suppression where the LOD score is above 95% experiment-wise significance. The light gray squares in the center of the dark gray bars represent a 1.0 LOD interval around the LOD peak for *slm1*, indicated by the black line through the light gray boxes.



ation of lesions by 26.6 days and reduced final lesion severity by 5.9 units. For both experiments, there is a significant correlation between initiation date and the final severity rating (Fig. 4).

For the MO01 experiment, a number of leaf coverage and rating observations were made after initiation. Figure 5 illustrates the increase in the coverage of the 10th leaf over time for two genotypes at the *slm1* locus as defined by genotype

at *bnlg1045*. The Mo20W/Mo20W genotype had only four individuals and was not considered in the analysis, although the progression curve for this genotype was very similar to the Mo20W/*les23* genotype. The rates of lesion growth between days 68 and 82 after planting were calculated for plants with Mo20W/*les23* and *les23/les23* genotypes at the *slm1* locus. Regression analysis was used to test whether rate of lesion progression was significantly different based on genotype. The rates of lesion growth were 3.82% leaf coverage per day for the *les23/les23* genotype and 1.38% leaf coverage per day for Mo20W/*les23* genotype and the difference in slopes was significant at $P < 0.0001$.

Discussion

The primary objective of this research was to identify suppressors of the *les23* lesion mimic phenotype by QTL analysis and therefore identify genetic loci capable of modifying cell death in maize. The bimodal distribution of final severity rating found in both the IA99 and MO01 experiments suggested a single factor significantly affecting the severity rating of *les23* (Fig. 2). Quantitative trait locus analysis identified one major factor, designated *slm1*, controlling the majority of the variation in lesion initiation timing. This QTL was subsequently shown to have a large effect in every model for lesion expression tested. These results indicate a strong correlation between the traits and suggest that initiation date is a determining factor for final severity. It is apparent that less severely expressing plants initiated lesions much later (Fig. 4). One Mo20W allele of *slm1* delayed expression of the *les23* phenotype for approximately 16 days for the IA99 experiment and 26 days for the MO01 experiment. That the allele responsible for lesion suppression comes from the Mo20W parent is consistent with previous observations of the Mo20W background acting as a suppressor to many lesion mimics and is also consistent with the observation that lesion timing is important for severity of lesion expression. It has been previously shown that Mo20W is able to suppress *Les1* in a dominant fashion through F₁ plants of a W23/*Les1* × Mo20W cross (Neuffer et al. 1983).

To further analyze the effect of *slm1* on the *les23* phenotype, two additional tests were performed. First, a method of comparing data was used that allowed the expansion of lesions or formation of new lesions over time along a leaf to be studied separately from the first initiation of lesions and final severity of the phenotype. The maximum slope of lesion coverage growth was calculated from a lesion growth curve of the lesion coverage on each individual plant's 10th leaf vs. date prior to day 88 as a non-initiation dependent measure of lesion progression and included in the QTL analysis for the MO01 experiment (rate of lesion expansion, Table 1). The removal of the major QTL effect was only partially successful because the effect of *slm1* was still significant, but with a lower amount of trait variation explained (Table 1). Second, percent leaf lesion coverage over time for the *slm1* genotype was tested for significance in the MO01 experiment. Regression analysis of the rate of lesion progression of *les23*/Mo20W vs. *les23/les23* phenotypes at the *bnlg1045* locus confirms that in addition to initiation, *slm1* also contributes to the reduction of lesion expansion rate (Fig. 5). It is possible that some of the increases in lesion

Table 1. Quantitative trait loci for lesion expression in two (Mo20W \times *les23*) F₂ experiments.

(a) Iowa 1999						
Trait	SSR Marker	Bin ^a	Additive	Dominance	R ² (%) ^b	LOD ^c
Lesion initiation ^d	<i>bnlg1045</i>	2.07	1.97	22.51	91.86	144.33
Final lesion severity ^e	<i>bnlg1429</i>	1.02	-0.57	-0.48	3.71	5.27
	<i>phi37</i>	1.08	-0.47	-0.52	2.53	4.57
	<i>bnlg1045</i>	2.07	-2.71	-2.07	73.08	108.91
	<i>phi328175</i>	7.04	0.64	0.30	2.17	4.41
Final percent lesion	<i>bnlg1429</i>	1.02	-5.89	-3.22	2.84	4.01
Coverage, 7th leaf	<i>bnlg1045</i>	2.07	-37.45	-15.63	75.45	89.79
Final percent lesion	<i>bnlg1429</i>	1.02	-6.57	-3.70	3.28	5.16
Coverage, 10th leaf	<i>bnlg1045</i>	2.07	-40.11	-16.71	77.44	101.66
(b) Missouri 2001						
Trait	SSR Marker	Bin ^a	Additive	Dominance	R ² (%) ^b	LOD ^c
Lesion initiation	<i>bnlg1045</i>	2.07	11.56	17.33	79.06	55.51
Final lesion severity	<i>bnlg1045</i>	2.07	-2.74	-3.57	87.39	88.25
	<i>bnlg1444</i>	4.08	1.01	-0.09	4.52	4.99
Rate of lesion expansion	<i>bnlg1045</i>	2.07	-1.38	-2.74	25.82	14.58
	<i>bnlg1444</i>	4.08	1.32	-0.32	8.10	4.62
	<i>umc1650</i>	4.09	1.57	-1.02	13.72	5.38
Area under the lesion	<i>bnlg2295</i>	1.04	-48.07	-4.50	2.70	3.65
Coverage curve	<i>bnlg1045</i>	2.07	-190.74	-190.71	83.41	77.78
	<i>bnlg1444</i>	4.08	75.37	5.55	6.65	6.91

^aPosition on the chromosome from MaizeDGB.^bPercent of the total phenotypic variation explained by the QTL.^cLog likelihood of a QTL being present vs. not present.^dDate after planting of first lesion expression.^eRating scale of 1–10 as described in the Materials and methods.

area were due to new lesion initiations near existing lesions rather than expanding lesions.

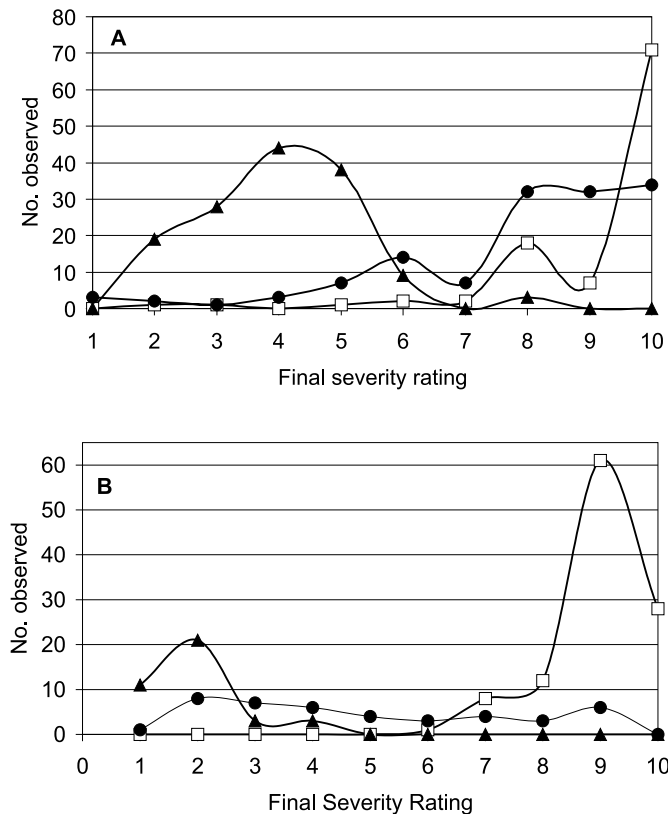
Although *slm1* was the only QTL detected for initiation date, several other QTL with smaller genetic effects were detected in various models of lesion coverage or final severity rating (Table 1). More QTLs were detected in the IA99 experiment than in the MO01 experiment. The IA99 experiment had nearly twice the individuals, which improves the power of detection of minor QTLs. Many markers significant in the IA99 experiment had LOD values near 3 in the MO01 experiment, but not high enough to meet the threshold LOD score set by permutation analysis. These minor QTLs did not affect lesion initiation, indicating that they acted to affect lesion severity only through modulation of lesion expansion. These additional factors may be partly responsible for the variation in final severity rating within the bimodal classes caused by *slm1*. It was observed in the field that some of the individuals in the MO01 and IA99 experiments had a more severe phenotype than the original *les23* parent, indicating a transgressive effect from one or more of the minor QTLs. These results suggest that these loci may be involved in the modulation of lesion expansion, but not initiation (Table 1). Therefore, QTL analysis was a successful approach for identifying factors that can modify two distinct steps in cell death, initiation, and lesion expansion (containment).

The *les23* mutant is a recessive mutation with characteristics similar to initiative class lesion mimics. Several *A. thaliana* mutants including, *hlm1*, *lsd4*, *lsd5*, and *lsd6*,

also show discreet lesions and more initiative-type lesion growth (Dangl et al. 1996; Balague et al. 2003). However, *les23* also shows some characteristics of propagative class mutants that grow over time to cover the leaf. Field observations show that necrotic spots of lesions sometimes continue to expand beyond purple borders that initially surround the dead tissue (Fig. 1, Panel B2). While the lesions grow, they do not expand as quickly or continuously as observed in the propagative mutant, *lls1* (Gray et al. 2002). Phenotypic expression of *les23* is dependent on light as has been previously observed in the *Les22* and *lls1* lesion mimics of maize, implicating the involvement of reactive oxygen species in the cascade of events leading to lesion formation (Hu et al. 1998; Gray et al. 2002).

What biological mechanism does *slm1* perform to alter the *les23* phenotype? Although many scenarios can be envisioned, a few examples are considered below, all of which are built on the assumption that *slm1* from Mo20W is superior to that of Va35. One possibility is that *slm1* from Mo20W encodes a gene product that effectively removes free radicals or limits their cytotoxicity. Such a function for *slm1* would not be noticeable until free radical production crosses a certain threshold, as may be the case in *les23*. Alternatively *slm1*, like *lsd1* of *Arabidopsis*, may encode a transcriptional regulator of genes that curtail cell death during stress responses, both biotic and abiotic (Dietrich et al. 1997; Eppele et al. 2003). Finally, *slm1* may behave like the barley *Mlo* gene, which, although not a transcriptional regulator per se, suppresses cell death initiated by free radicals (Buschges et

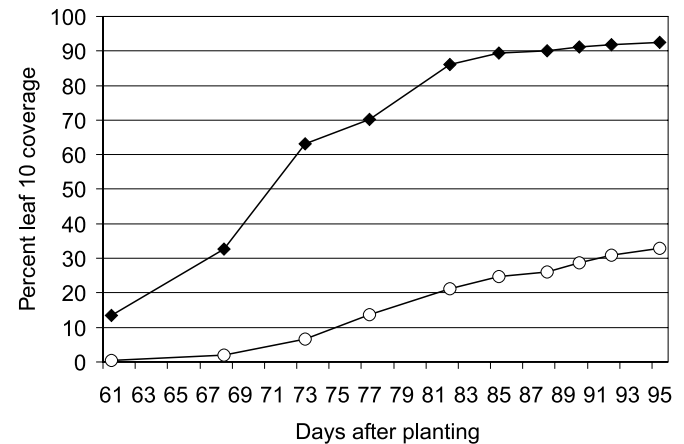
Fig. 4. Distribution of final severity rating by initiation date in two QTL experiments. Each line represents a range of initiation dates where newly expressing plants were discovered. The filled triangles indicate late initiating plants, the open squares indicate early initiating plants, the filled circles indicate moderately late initiating plants. (A) IA99 experiment, 374 individuals. (B) MO01 experiment, 189 individuals.



al. 1997; Piffanelli et al. 2002). The light dependence of *les23* suggests that *slm1* may function by suppressing free radicals. Since many other maize lesion mimics require light for manifestation, the cell death suppressing ability of *slm1* may extend to these mimics as well. In line with this idea is the observation that Mo20W provides a suppressible background for a number of maize lesion mimic mutations (Neuffer et al. 1983).

Disease lesion mimics are useful for studying the effects of stresses on plants, including cell death responses to invading pathogens, without the complications of the presence of pathogens. The QTL method used here allowed for a quick screening of the entire genome for candidate loci involved in and impacting cell death. Because *slm1* has such a major effect on the *les23* phenotype, it should provide an excellent system to study the molecular basis of lesion development. It is important to determine whether the *slm1* locus can affect multiple lesion mimics as well as plant responses to other abiotic and biotic stresses. Since lesion mimic mutations have often been associated with enhanced disease resistance (Arase et al. 2000; Yin et al. 2000; Jambunathan et al. 2001; Balague et al. 2003), one potentially novel use of this locus could be to modify tolerance to pathogens for which maize has poor resistance.

Fig. 5. Lesion progression curves for two genotype classes at *slm1*, the major QTL in the MO01 experiment. Each line corresponds to the averaged value of percent lesion coverage on the 10th leaf for all plants of genotype, *les23/les23* (filled diamonds), or Mo20W/*les23* (open circles), at SSR marker *bnlg1045* on a particular date shown as days after planting.



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